1496

Protection Branch Report of Test No. 13-62

Sterilization

INVESTIGATION OF MICROBIAL CONTAMINATION INSIDE CURED SOLID PROPELLANT

8 November 1961

Prepared by:

Dorothy M. Portner
DOROTHY M. PORTNER
Decontamination Section
Protection Branch

Approved by:

Robert K Hoffman
ROBERT K. HOFFMAN
Chief, Decontamination Section
Protection Branch

Herbert M Decker
HERBERT M. DECKER
Chief, Protection Branch

Charles R Phillips
CHARLES R. PHILLIPS
Chief, Physical Defense Division

Physical Defense Division
Fort Detrick, Frederick, Maryland

Protection Branch Report of Test No. 13-62

Investigation of Microbial Contamination Inside Cured Solid Propellant

Cured solid propellant received in August 1961 from Thiokol Chemical Corporation, Elkton, Maryland, was tested for possible internal microbial contamination. The purpose of testing the propellant was to determine whether treatment would be necessary in order to comply with the recent NASA policy on the sterility of extraterrestrial impact spacecraft. The propellant in question will be used in the retrorocket designed to slow down a Surveyor spacecraft as it approaches the lunar surface. In event of a hard landing caused by failure of the retrorocket, the unburned propellant could contaminate the moon if viable microorganisms were present. Microbial contamination was present inside a finished solid propellant previously tested 1/. The investigation to determine whether Thiokol cured solid propellant also has internal microbial contamination is the subject of this report.

MATERIALS AND METHODS

Cubes of cured solid propellant (about 1/2 x 1/2 x 1/2 inch), scapels, forceps, and fluid thioglycollate medium blanks sealed with tape were placed in a plastic chamber 2/ and exposed to ethylene oxide gas for six hours. After aerating the chamber for 16 hours, each cube of propellant was minced and the pieces put into a fluid thioglycollate medium blank to incubate at 37C. In addition to mincing the propellant to determine internal microbial contamination, one unminced cube per test was put into fluid thioglycollate medium to determine whether the exterior surfaces were sterilized in the prescribed six hour ethylene oxide exposure period. After the samples had incubated at 37C for seven days, an aliquot of each sample was streaked on tryptose agar to check for microbial growth. A methylene blue stain of each sample was examined microscopically for microorganisms and compared with a stain of the microorganisms from agar if growth occurred. Finally, if no microorganisms grew on agar or if no organisms were seen during examination of the methylene blue stain of the fluid thioglycollate sample, the sample was inoculated with 20 to 100 spores of Bacillus subtilis var niger to assure that the medium was capable of supporting bacterial growth.

RESULTS AND DISCUSSION

No microbial contamination was evident in any of the 37 cubes of cured solid propellant tested. The external surfaces of all the cubes of propellant tested were sterile after exposure to ethylene oxide gas for six hours. After B. subtilis var niger spores were introduced into the sample, bacterial growth was always evident in the medium containing a whole cube of propellant; but bacterial growth frequently was not evident in the medium containing minced propellant.

This inhibitory property of the propellant was evident in 14 out of 28 minced samples. The inhibitory properties could be overcome, however, by a threefold dilution of the sample. Subsequent tests therefore included the following additional step in the test procedure. After the minced propellant had been added to the fluid thioglycollate blank, two aliquots of the sample were diluted threefold with fluid thioglycollate medium and incubated at 37C for seven days. No microbial growth was evident in any of the nine propellant samples or their corresponding subcultures. However, only the subcultures supported growth of B. subtilis var niger that was deliberately introduced into the samples later.

Although this propellant in broth will inhibit growth of microorganisms, there is no indication that it will also kill them. Spores of B. subtilis var niger were added to a sample of minced propellant and distilled water; then the sample was assayed by the pour plate method immediately after the spores were added to the sample and again after a 24 hour contact period. No bacterial reduction was evident even after 24 hours contact. Tests with minced laboratory-made samples of propellant in fluid thioglycollate medium also indicated that the propellant was not bactericidal. The laboratory-made propellant, unlike the propellant made for production, had a wax-coated cardboard backing attached to one side which was contaminated with microorganisms. Bacterial growth was evident in seven out of ten of these samples one day after placing the minced material in broth; and after a seven day incubation period, a high concentration of viable microorganisms was present in all seven samples.

The possibility that the interior of the propellant might have been sterilized by the ethylene oxide gas treatment was also investigated. Although ethylene oxide gas did not sterilize the cardboard backing, it might be possible to sterilize the interior of the small propellant cubes since the latter is a porous material. A needle and

syringe was used to insert a B. subtilis var niger spore suspension into cubes of propellant and the cubes were then allowed to dry at room temperature for several days before exposure to ethylene oxide gas for six hours. The propellant was then minced, put into fluid thioglycollate medium, and aliquots of the sample subcultured. None of the undiluted samples showed evidence of bacterial growth, but bacterial growth was present in the subcultures. The results indicate therefore that the ethylene oxide treatment used to sterilize the exterior did not penetrate the propellant and sterilize the interior.

Although no viable microorganisms were found in the few samples tested it cannot be stated categorically that all of this particular propellant is sterile. It is recommended, however, that further tests be conducted deliberately adding viable bacterial spores to the product during processing and then determine whether the material self-sterilizes. If the propellant is self-sterilizing, it would alleviate the necessity of applying heat, radiation or other treatment in order to comply with the requirement for sterilization as set forth by the National Aeronautics and Space Administration.

References

1. Protection Branch Report of Test No. 14-61 "Investigation of Microbial Contamination Inside Balsa Wood and Explosive Charges (Squibs, Pyrotechnic Pellets and Finished Propellant)", 19 May 1961.

2. Protection Branch Report of Test No. 7-60 "A Technique for the Investigation of Bacterial Contamination Inside Electronic Components" 11 March 1960.